Supporting Material:

A Molecular Trajectory of α-Actinin Activation

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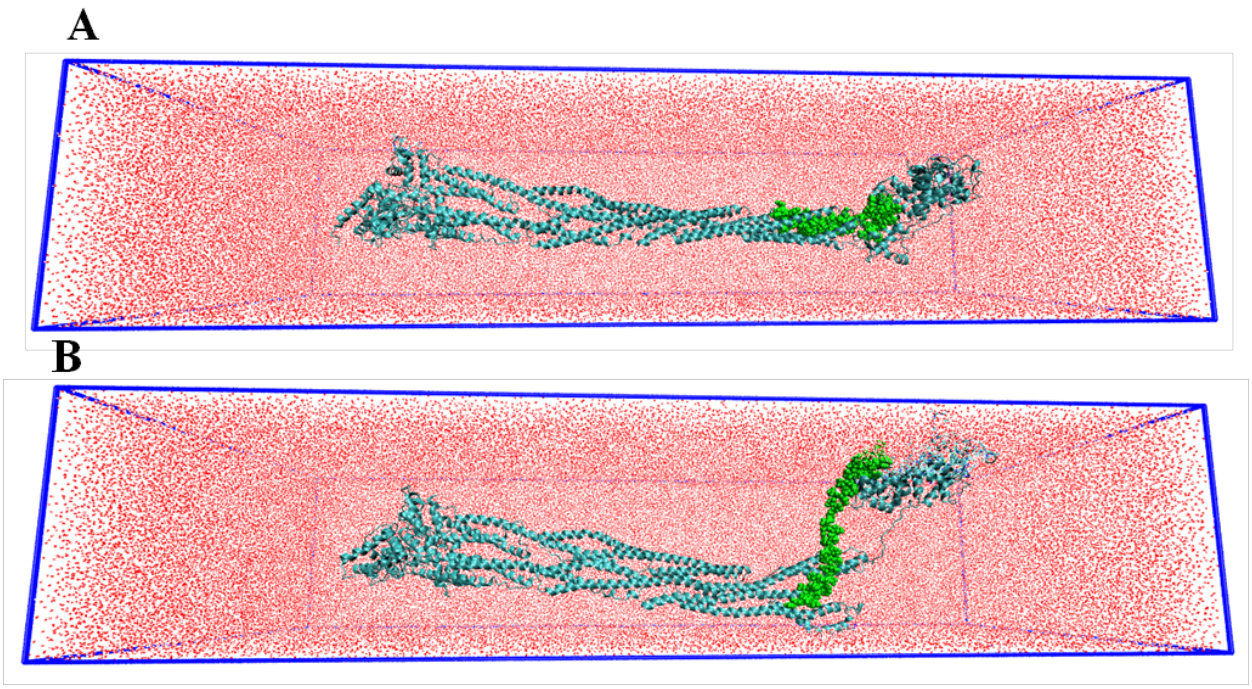

FIGURE S1. The confined structure of α-actinin in the water box (A) before and (B) after the activation. The minimum distance between the closest atoms at the top and bottom side of the molecule in the simulation box is approximately 13 Angstroms, which is larger than the cutoff distance of non-bonded interactions (10 Angstroms). Some of the water molecules are cut out from the front side of the box for the sake of clarity.

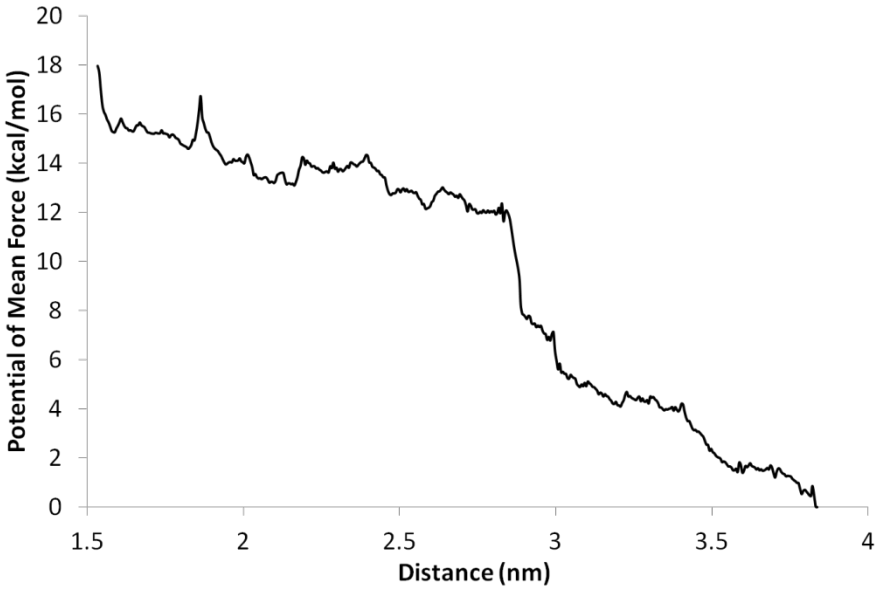

FIGURE S2. The potential of mean force calculated with a higher umbrella potential of 4000 kJ/mol nm^2. The downhill behavior of the PMF as well as the point of activation is conserved.

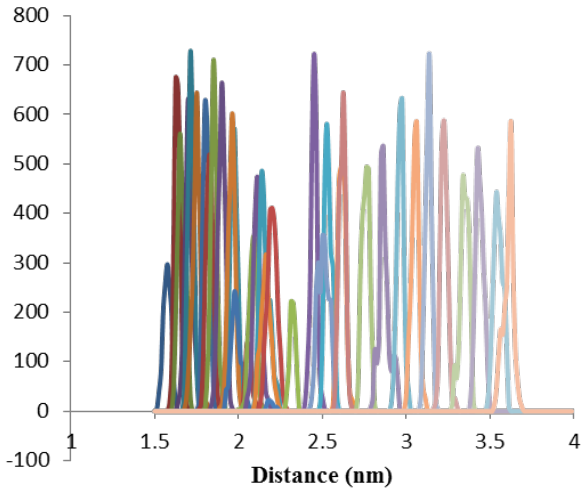

FIGURE S3. The histograms generated in the umbrella sampling simulations along the reaction coordinate. They show a good overlap. This condition guarantees a good sampling of the system and a smooth potential of mean force.

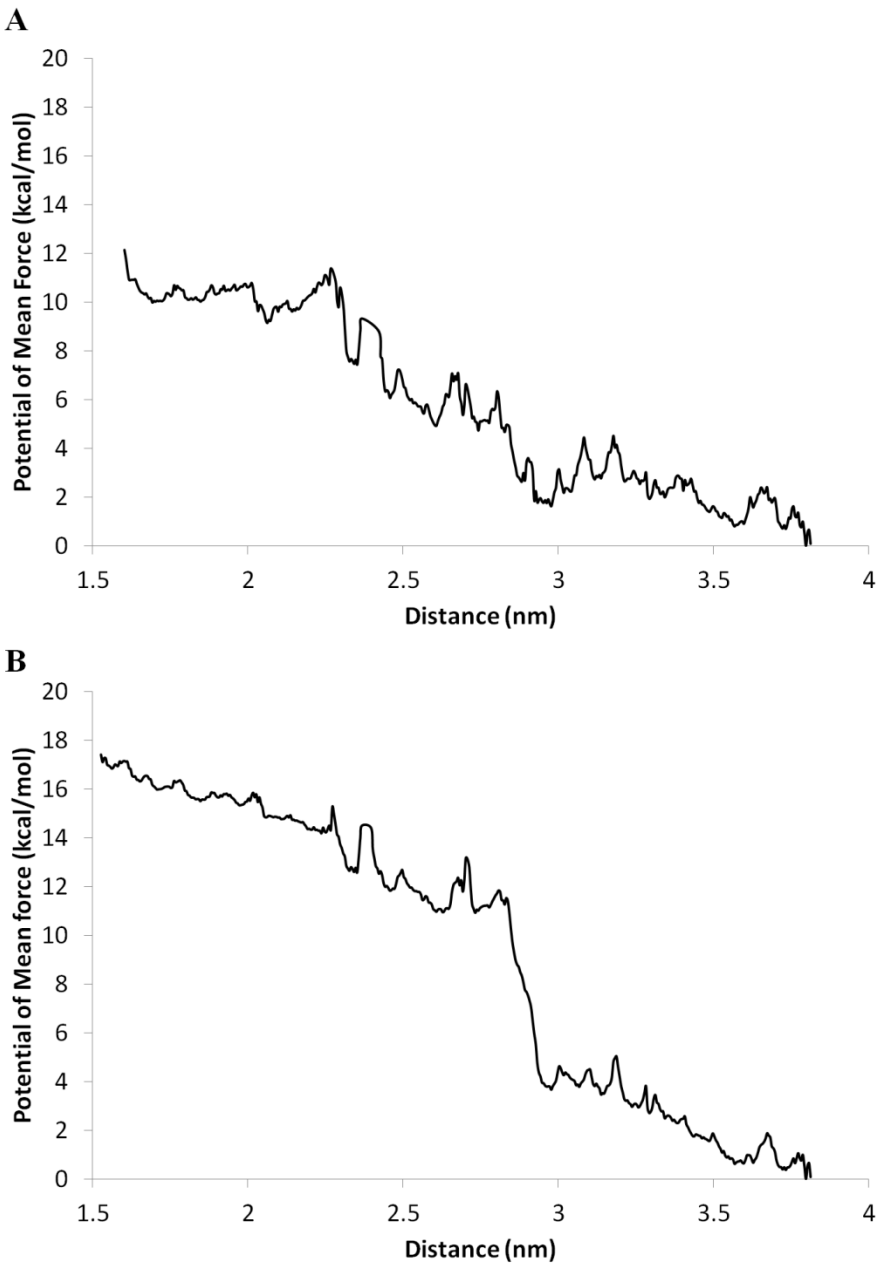

FIGURE S4. The potential of mean force calculated with different number of data points. (*A*) Keeping 30% of the original data points resulted in a noisy graph. (*B*) Keeping 80% of the data points resulted in a PMF close to the original one showing that the sampling was good enough.

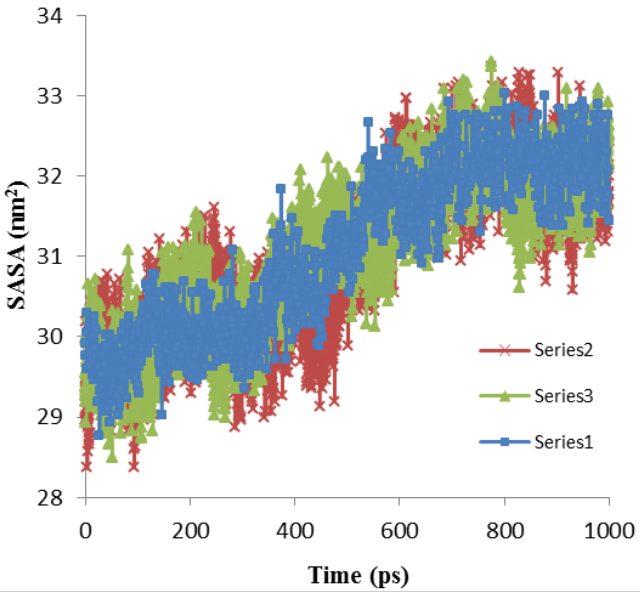

FIGURE S5. The effect of salinity on the solvent accessible surface area of αVBS was measured and compared to the simulation with no salt concentration. In the original simulation, system was only neutralized and no extra salt was included. Addition of 50 mM and 150 mM salt to the system affected neither the trend nor the rate of activation process, most likely because the dominant forces in inhibiting αVBS are hydrophobic in nature.

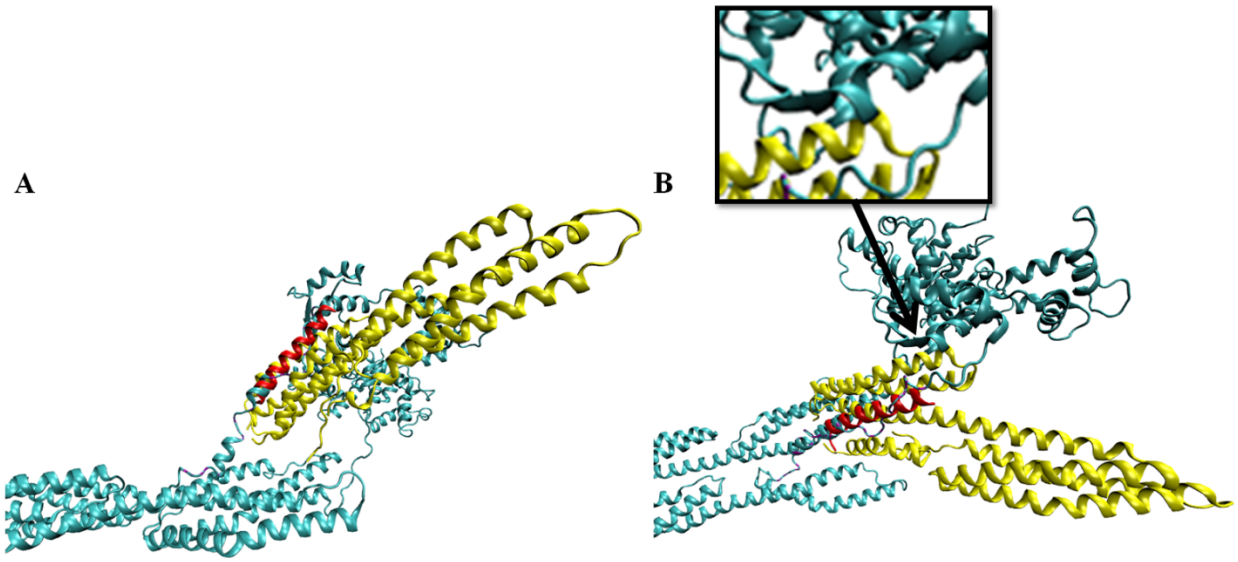

FIGURE S6. Activated structure of α-actinin (cyan) aligned to the vinculin (yellow)-αVBS (red) complex. (*A*) The side view of the aligned structures depicts the relative position of vinculin head with respect to αVBS. (*B*) The up view of the same configuration as S6*A* shows a plausible consistency of structures other than a small overlap of vinculin with the actin binding domains of α-actinin marked by the black arrow (this region is magnified in the insert). This overlap is not important since the vinculin- αVBS structure was directly taken from the PDB bank and not equilibrated, therefore, producing some bad contacts in the system was expected.

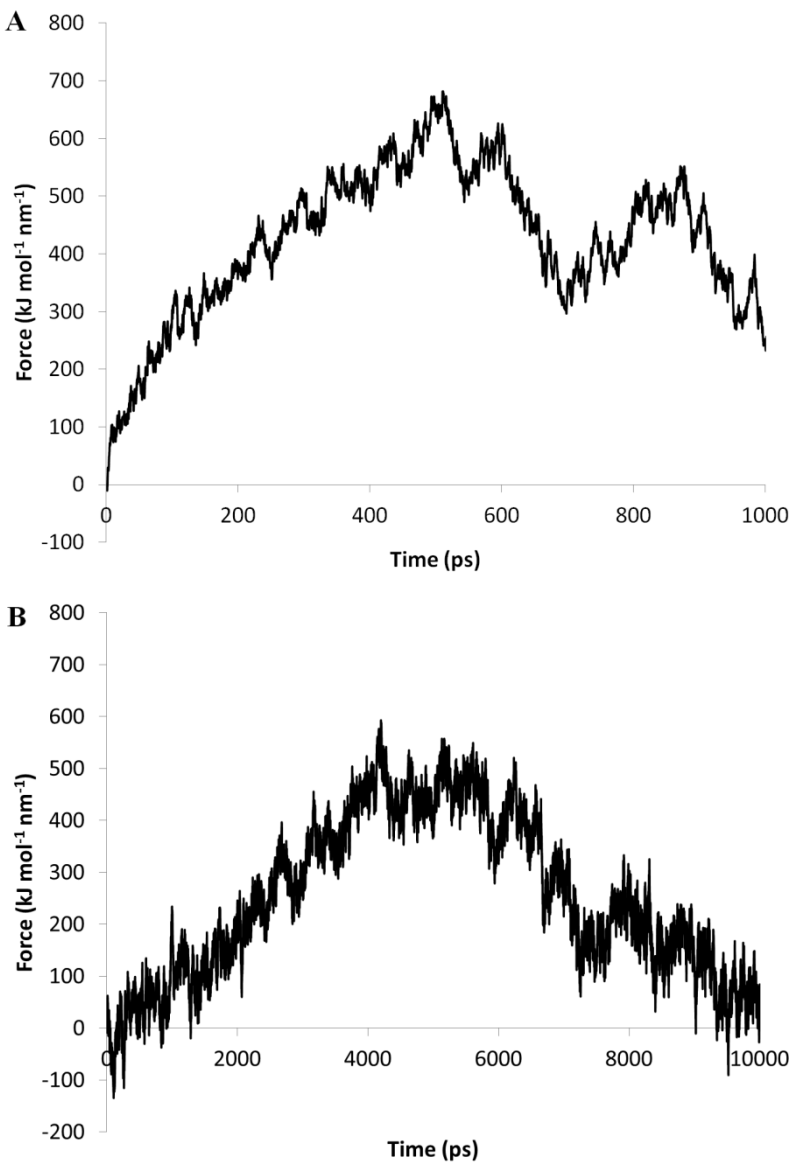

FIGURE S7. Force applied to αVBS during activation trajectories generated with different pulling velocities. (*A*) For the pulling velocity of 0.005 nm/ps, the maximum value of force was 650 kJ/(mol nm) that ocurred at 500 ps where the activation process was completed. (*B*) Using a lower pulling velocity of 0.0005 nm/ps, the maximum value of force was 20% less than the original force but the peak was again at the midway of the trajectory.

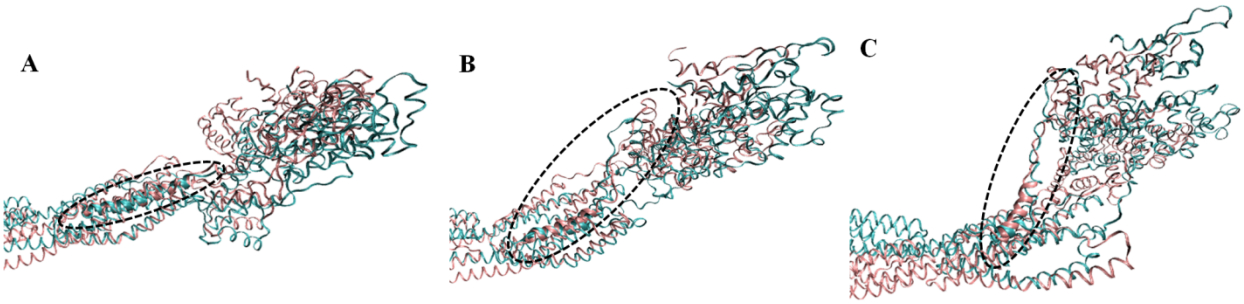

FIGURE S8. Aligned structures of α-actinin from the original (pink) and low-velocity (cyan) trajectories at three similar stages of activation: A) After 10% of both trajectories passed αVBS was still in the inactive conformation; (B) At the midway of both trajectories, αVBS was halfway exposed; And (C) after 90% of trajectories passed complete activation was reached. Dashed ellipse marks the position of αVBS at each time-point showing that αVBS followed similar activation pathways in both trajectories. Therefore, we expect that performing umbrella sampling calculations on both trajectories result in the same potential of mean force.

Table S1

Residue in contact with αVBS	**αVBS**	**Final Interaction**
ILE647	GLY717	ILE679
MET651	GLN720	ILE679
ILE654	THR724	TYR675
ILE689	MET711	ALA640
ILE686	ILE714	ILE647
TYR682	TRP718	TRP646
ILE679	LEU721	ILE647
GLU676	ILE725	ILE654
VAL329	GLU719	TRP369
GLN334	ALA726	LEU283
GLU336	ARG727	VAL362

Residues in contact with αVBS form new interactions after activation. The final interactions are mostly hydrophobic in nature and responsible for the stability of the system after activation.